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Identifying genetic regulators of cancer stem cell differentiation for glioblastoma therapy
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Abstract

Background:
- Glioblastoma multiforme (GBM) is the most aggressive and common type of primary brain cancer. Poor therapeutic outcomes may be attributed to the presence of a subpopulation of cells, known as cancer stem cells (CSCs). CSCs are capable of sustained self-renewal, differentiation into terminal cell types, and chemotherapy resistance.

Results:
- Single-cell RNA-sequencing (scRNA-seq) data for 18636 genes in 1867 cells was analyzed. Populations of CSCs, neurons, astrocytes, and oligodendrocytes were identified from clustered cells. 21 genes were identified as negative regulators of CSC differentiation into all three differentiated cell types. Central nervous system (CNS) development and drug response were the most significantly enriched biological processes from gene ontology analysis.

Conclusions:
- Unsupervised clustering of scRNA-seq data from patient-derived GBM neurospheres reveals subpopulations of cells with distinct genetic signatures. Pseudotime-ordering trajectory can be used to determine genetic regulators of CSC differentiation. Further work is necessary to analyze scRNA-seq data from other GBM samples and select candidate genes for therapeutic validation.

Introduction

Figure 1: CSC-directed therapies may increase GBM sensitivity to chemotherapy and improve patient outcomes. (a) CSCs are resistant to conventional cancer therapies and continue to proliferate and differentiate into tumor cells after treatment. (b) Selectively differentiating CSCs within the tumor into chemoresistant-sensitive tumor cells can increase therapeutic efficacy of conventional cancer therapies. Adapted from Lathia, et al. at Genes Dev. (2015)

Methods

Figure 2: Workflow of scRNA-seq analysis to identify genetic regulators of CSC differentiation. (a) Quality control, data normalization, linear correction, dimensionality reduction, and clustering for 18636 genes in 1867 cells was performed using Seurat. (b) Single-cell trajectory construction and analysis of branches in pseudotime was performed using Monocle. (c) Gene ontology analysis, visualization, and interpretation was performed using The Database for Annotation, Visualization, and Integrated Discovery (DAVID), Cytoscape, and Enrichment Map.

Figure 3: Unsupervised clustering of scRNA-seq data from patient-derived GBM neurospheres. (a) t-distributed stochastic neighbor embedding (tSNE) plot highlights genetic differences among single cells using Seurat. The distance between any two cells reflects the similarity of their expression profiles. Clusters are used to identify cell types. (b) Heatmap of top ten gene markers for each cluster from the tSNE plot in (a). CSCs that are partially differentiated may be spread across multiple clusters. Differentiated CSCs (astrocytes, neurons, and oligodendrocytes) will be more isolated. (c) Number of genes. (d) Percent of mitochondrial genes. (e) Number of unique molecular identifiers. (f-e) Heatmap from batch A. Blue represents cells from batch B. Both batches are from same patient-derived GBM neurospheres.

Figure 4: Identifying cell types from scRNA-seq data using patient-specific and canonical gene markers. (a-d) Violin plot showing average gene expression of patient-specific gene marker for each cell type. (a) STAT3 is used as the CSC gene marker. (b) STMN2 is used as the oligodendrocyte gene marker. (c) PLF1 is used as the astrocyte gene marker. (e-h) Box-and-whisker plots showing average gene expression of canonical gene markers for each cell type. (e) GRIA1, OLIG2, ITGA6, POGFRA, DLL3, and OLIG1 are used as the CSC gene markers. (f) NEFL, RIBOFX3, GRIA3, STMN2, COBL, LIT1C, DCX, and CD24 are used as the neuron gene markers. (g) GFAP, PAGQ6, PPP1R3C, C16orf99, ALDC2, and AP2G4 are used as the astrocyte gene markers. (h) MLN, PLP1, MBP, MAG, and MOG are used as the oligodendrocyte gene markers. Colors correspond to fig. 3a cluster colors.

Figure 5: Pseudotime-ordering trajectory replicates CSC differentiation to terminal cell types. Gene ontology analysis shows enrichment for central nervous system development

Gene ontology analysis shows enrichment for central nervous system development

Figure 6: Gene ontology analysis of genes that negatively regulate CSC differentiation. (a) Venn diagram of genes that negatively regulate CSC differentiation into each of the terminal cell types. q-val < 1e-5. (b) Significant biological processes determined using DAVID. Multiple test correction was performed using the Benjamini-Hochberg correction. (c) Gene ontology bubble plot of genes in (a). q-val < 0.05. Nodes represent DAVID annotation terms. Node size is positively correlated with the number of genes in each annotation. Enriched biological processes from (b) are indicated by the colored ellipsoids. * < 0.05. ** < 0.01